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Published in:
Organic letters

DOI:
[10.1021/ol0507524](https://doi.org/10.1021/ol0507524)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

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Citation for published version (APA):

West, K. R., Bake, K. D., & Otto, S. (2005). Dynamic Combinatorial Libraries of Disulfide Cages in Water. *Organic letters*, 7(13). <https://doi.org/10.1021/ol0507524>

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Supplementary Information

Dynamic Combinatorial Libraries of Disulfide Cages in Water

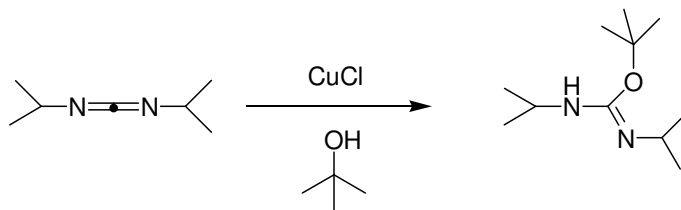
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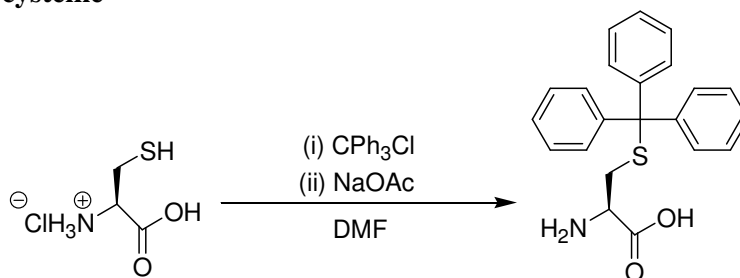
I. Synthetic Procedures for 5

N,N'-diisopropyl-*O*-*tert*-butylisourea^A



In an oven dried flask a mixture of 511 mmol of 1,3-diisopropylcarbodiimide (80.0 mL), 594 mmol of *tert*-butanol (distilled off CaH₂) (56.0 mL) and 0.5 mmol of CuCl (0.50 g) was prepared. The dark green suspension was stirred under nitrogen for 5 days before a ¹H NMR was recorded to check that the reaction was complete. To obtain a CuCl free sample, a small amount of the neat suspension was filtered into an NMR tube and dissolved in CDCl₃. When the reaction was complete the *N,N'*-diisopropyl-*O*-*tert*-butylisourea was distilled under vacuum to give a colourless liquid (103.2 g, 98%, mixture of *cis* and *trans* isomers). (bp 86°C, 0.24 mm Hg). ¹H NMR (CDCl₃) δ 3.70 and 3.62 (sept_{cis}, sept_{trans}, 1H, J = 6.3, 6.5) 3.19 and 3.10 (brsept_{cis}, sept_{trans}, 1H, J = 6.2) 1.44 and 1.34 (s_{cis}, s_{trans}, 9H) 1.05 (d, 6H, J = 6.4) 1.01 (d, 6H, J = 6.2).

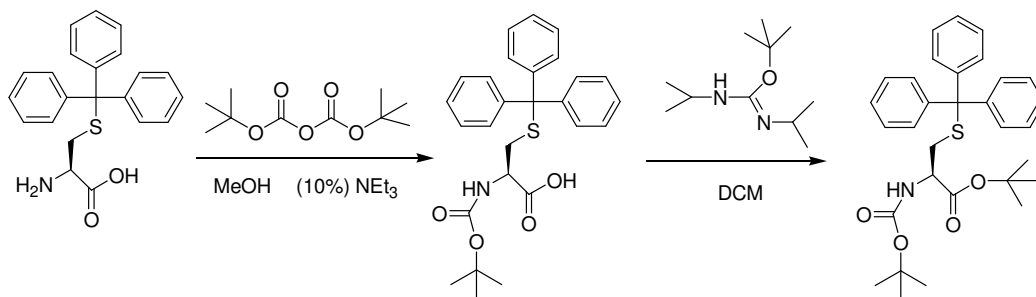
S-Trityl-L-cysteine^B



L-Cysteine hydrochloride monohydrate (10.0 g, 63 mmol) and trityl chloride (27.0 g, 63 mmol) were stirred in 40 mL DMF for 2 days at room temperature. A 10% sodium

acetate solution (350 mL) was added and the precipitate was filtered and washed with distilled water. The filtrate was stirred in acetone at 50°C for 30 min and filtered after cooling. The residue was then washed with acetone and diethyl ether. After drying in vacuo, 20.5 g of trityl cysteine was obtained as a white powder (89%): mp 195°C (decomposition); ¹H NMR (DMSO-d₆) δ 2.49 (dd, J = 9 Hz, 12Hz 1H CH₂) 2.58 (dd, J = 4.4 Hz, 12 Hz, 1H, CH₂) 2.91 (m, 1H, CH) 7.22-7.36 (m, 15H, arom H).

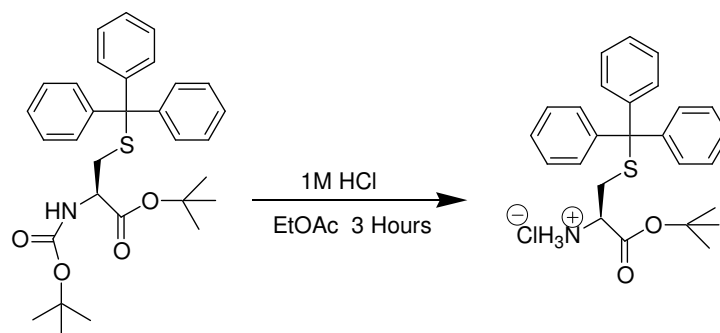
***N*-BOC-*S*-trityl-*L*-cysteine-*O*-*tert*-butyl ester (2)^{C,9}**



S-trityl-*L*-cysteine (28 mmol, 10.0 g) was dissolved in 80 mL of anhydrous 10% NEt₃ (distilled of CaH₂) in MeOH with vigorous stirring. When dissolution was complete, 55 mmol of di-*tert*-butyl dicarbonate (12.0 g) was added. The solution was refluxed under a nitrogen atmosphere for 1.5 hours and the reaction was monitored by TLC, (silicagel; CHCl₃/MeOH 12:1; R_f trityl cysteine = 0; R_f product = 0.47 (streaks); R_f di-*tert*-butyl dicarbonate = 0.88). The solvent was evaporated to give a foaming oily residue, which was redissolved in ice cold HCl (pH 2.2, 80 mL) This solution was stirred for 10 minutes and extracted at least four times with 400 mL of ethyl acetate. Extraction was continued until no more residue was extracted from the solution as evident upon evaporation of solvent. The extracted product was dried with MgSO₄, and the solvent evaporated to give a foaming oily residue which was dried with under vacuum for 3 hours (TLC silicagel; CHCl₃/MeOH 12:1; R_f product = 0.30).^C

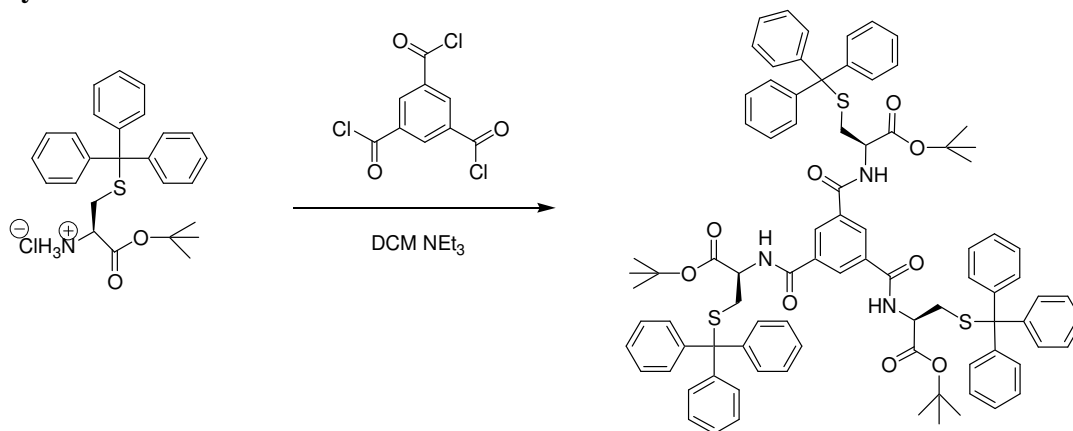
The residue was redissolved in 20 mL of dry DCM and 83 mmol (16.5g) of the *N,N'*-diisopropyl-*O*-*tert*-butylisourea was added. The resultant yellow solution was stirred and refluxed for 24 hours at which point the diisopropylurea side product had precipitated. (TLC silicagel; Hexane/Et₂O 2:1; R_f product = 0.45; R_f side product = 0.16; R_f starting material = 0). The *N*-BOC-*S*-tritylcysteine *tert*-butyl ester was then filtered through a silica plug using 2:1 hexane/diethyl ether. The solvent was evaporated to produce a foaming colourless oil which was dried to give the desired product as a white solid (yield over two steps: 8.9 g, 61% [NB yield of *tert*-butylation of commercial *N*-BOC-*S*-trityl cysteine: 90%]) ¹H NMR (CDCl₃) δ 7.43-7.19 (m, 15), 5.10 (brd, 1H, J = 8.1), 4.22-4.19 (brm, 1H), 2.52 (brd, 1H, J = 4.5), 1.46 (s, 9H), 1.43 (s, 9H).

***S*-Trityl-L-cysteine-*O*-*tert*-butyl ester (3)**¹⁰



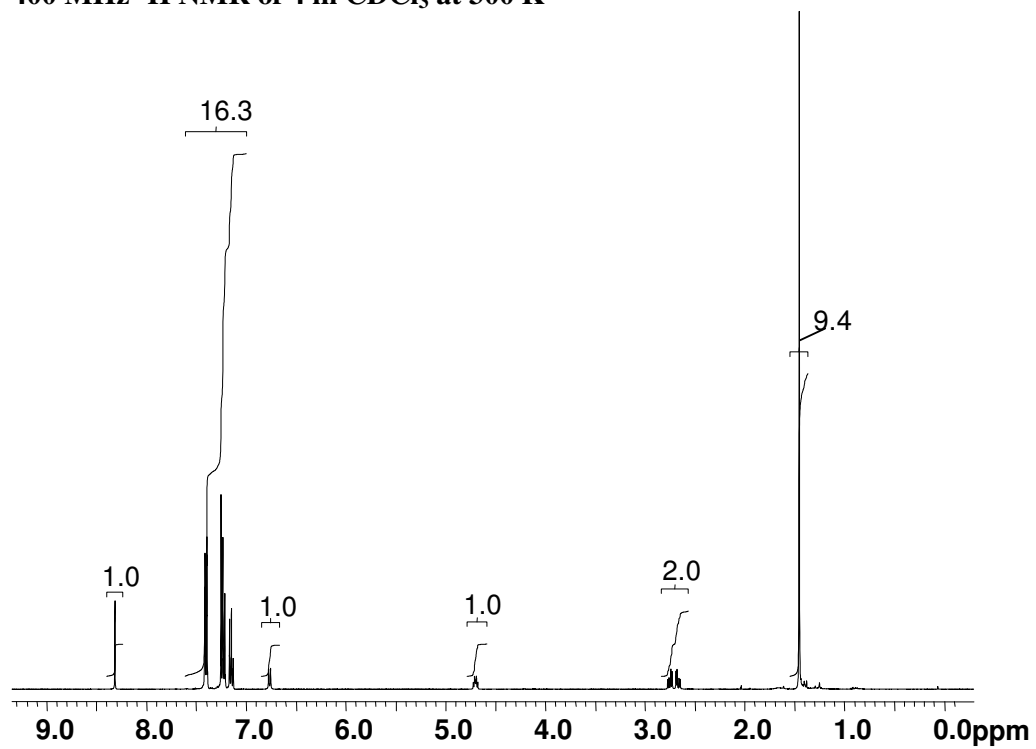
Acetyl chloride (58 mmol, 4.1 mL) was dissolved in 58 mL anhydrous ethyl acetate. Anhydrous methanol, 58 mmol (2.4 mL) was added dropwise and the solution was stirred for 20 minutes at room temperature under an inert atmosphere. *N*-BOC-*S*-trityl-L-cysteine-*O*^{*t*}Bu ester, 12 mmol (6.0 g) was added to the HCl solution via a solid addition side arm. The solution was stirred for 3 hours at room temperature before being analysed by TLC (silicagel; CHCl₃/EtOAc/MeOH; 15:5:1; R_f_{starting material} = 0.94 R_f_{product} = 0.63 R_f_{tritylcysteine} = 0). The solution was evaporated to give a (foaming) white residue and triturated with anhydrous diethyl ether. Dry flash chromatography was used to recover the unreacted starting material and purify the *S*-trityl-L-cysteine-*O*^{*t*}Bu ester hydrochloride. The starting material was recovered by eluting through the silica plug using 2:1 hexane/diethyl ether until no more material was detected by TLC and the pure *S*-trityl-L-cysteine-*O*^{*t*}Bu ester hydrochloride was obtained by eluting with DCM/EtOAc/MeOH; 15:5:1. The reaction was then repeated using the unreacted starting material. Yield: 3.9 g, 71%, recovered starting material: 0.9 g, 18%. ¹H NMR (MeOD) δ 7.43-7.21 (m, 15H), 3.20 (dd, 1H, J = 5.5, 6.5), 2.66 (dd, 1H, J = 7.2, 13.1) 2.61(dd, 1H, J = 5.4, 13.1) 1.42 (s, 9H). ¹³C NMR (MeOD) 169.8 (ester carbonyl), 145.5 (trityl, C adjacent to quaternary C), 130.6 (trityl), 129.2 (trityl), 128.2 (terminal trityl C), 84.7 (quaternary *tert*-butyl C), 68.4 (quaternary trityl C), 54.2 (α-amino C), 34.7 (thiol methylene C), 28.2 (terminal *tert*-butyl C). Exact mass: calcd: 420.1997; found: 420.2009 (M+H⁺). Decomposition temperature range: 64-73°C.

Synthesis of 4¹¹

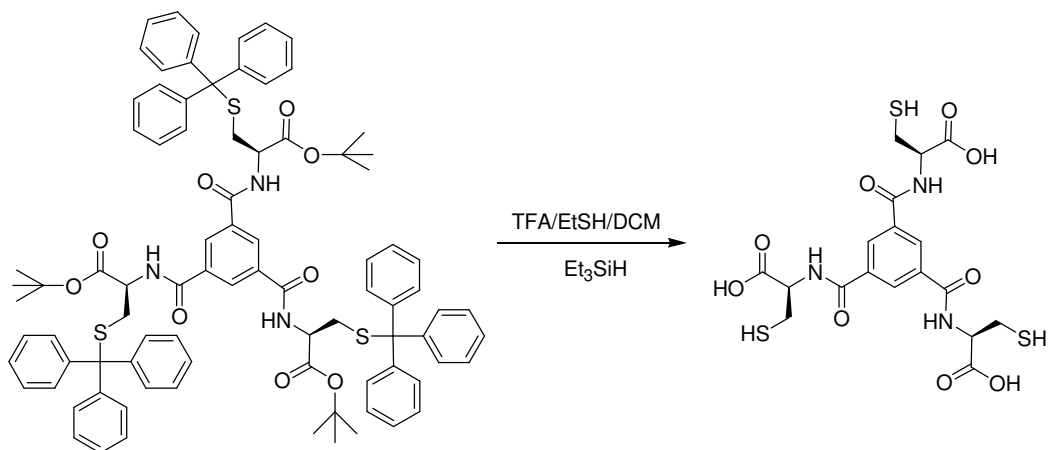


S-trityl-L-cysteine-*O*^tBu ester hydrochloride, (3.6 mmol, 1.6 g) was dissolved in 15 mL of anhydrous DCM under an inert atmosphere. The solution was cooled to 0°C and 8.9 mmol (1.2 mL) of dry NEt₃ (distilled off CaH₂) was slowly added while stirring, causing the mixture to turn opaque. The solution was left to stir for 20 minutes before gradually adding the acid chloride (0.89 mmol = 0.263 g). The solution was left to stir in the melting ice bath under N₂ for 20 hours. TLC; silicagel; DCM/EtOAc; 3:1; R_f_{product} = 0.95. The solution was filtered to remove the precipitate and successively washed with 2x 20 mL 1.4M HCl, 2x 20 mL saturated sodium carbonate solution (note, can form a stable suspension if shaken too vigorously) and 20 mL water. The organic layer was then dried over magnesium sulfate and purified by dry flash chromatography, eluting with DCM/EtOAc; 3:1. The product was dried under vacuum to give a foaming white solid (yield: 0.6 g, 47%). ¹H NMR (CDCl₃) δ 8.31 (s, 3H) 7.41-7.13 (m, 45H) 6.76 (d, 3H, J = 8.0) 4.70 (ddd, 3H, J = 4.8, 5.8, 7.8) 2.75 (dd, 3H, J = 5.8, 12.3), 2.67 (dd, 3H, J = 4.8, 12.3) 1.45 (s, 27H). ¹³C NMR (CDCl₃) 169.1 (ester carbonyl), 164.9 (amide carbonyl), 144.3 (trityl, C adjacent to quaternary C), 134.8 (central aromatic C adjacent to amide), 129.5 (trityl), 128.8 (central aromatic CH), 128.0 (trityl), 126.8 (terminal trityl C), 82.9 (quaternary *tert*-butyl C), 66.8 (quaternary trityl C), 52.4 (α-amino C), 34.1 (methylene C), 28.0 (terminal *tert*-butyl C). Exact mass: calcd: 1436.5502; found: 1436.5497 (M+Na⁺). Decomposition temperature range: 128-144°C.

400 MHz ^1H NMR of 4 in CDCl_3 at 300 K

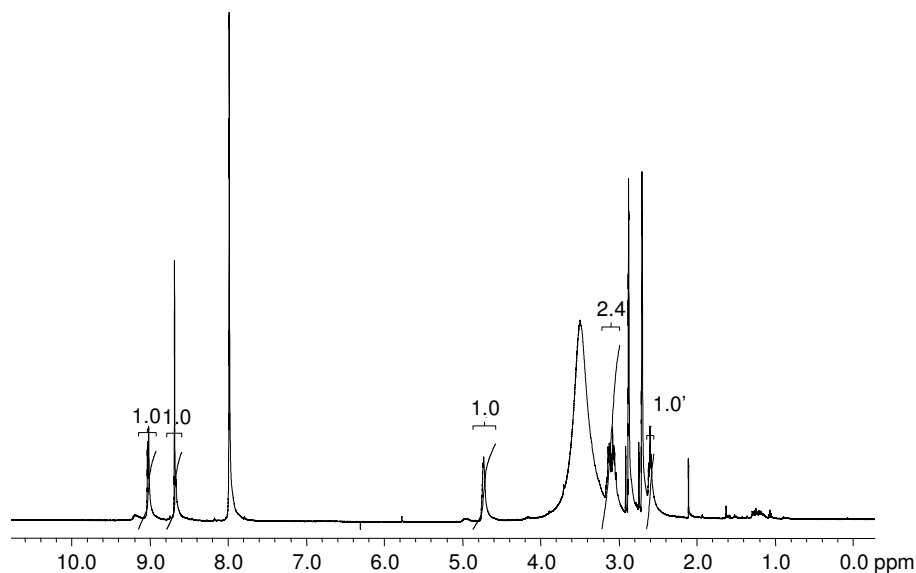


Trithiol **5**¹²



Benzene-1,3,5-tricarbonyl-*S*-trityl-L-cysteine-*O*^tBu ester, 0.707 mmol (1.00g) was dissolved in a solution of 10mL degassed TFA, 10mL ethanethiol and 5mL degassed DCM. A bright orange solution was formed which was stirred under N₂ for 4 hours before adding 1.061 mmol (0.17mL) of triethylsilane. Soon after addition, the solution became colourless and the stirring was continued for an hour under N₂. At this point, the solvents were evaporated and the residue was stirred with 200mL of degassed MilliQ water for 30 minutes. The resulting solution was filtered under N₂ and successively washed with 3x30 mL chloroform and 3x30mL diethyl ether. The water was evaporated under vacuum to give the product as a white residue (0.21g, 58%) ¹H NMR (DMF) δ 9.02 (brd, 3H, J = 7.5) 8.68 (s, 3H) 4.75-4.71 (brm, 3H) 3.16-3.03 (brm, 6H) 2.60 (brt, 3H, J = 8.6). ¹³C NMR (DMF) δ 172.3 (carboxylic acid carbonyl), 166.7 (amide carbonyl), 135.5 (central aromatic C adjacent to amide), 129.9 (central aromatic CH), 56.9 (α-amino C), 26.0 (methylene C). Exact mass: calcd: 518.0362; found: 518.0367 (M-H⁺). Decomposition temperature range: 209-214°C.

500 MHz ¹H NMR of **5** in *d*⁷-DMF at 300 K



Additional references

- A. (i) Hayen, R. Koch, W. Saak, D. Haase, J. O. Metzger, *J. Am. Chem. Soc.* **2000**, *122*, 12458. (ii) R. G. Ball, G. D. Berger, *J. Org. Chem.* **1994**, *59*, 2261.
- B. J. Rudolph, H. Theis, R. Hanke, R. Endermann, L. Johannsen, F.-U. Geschke, *J. Med. Chem.* **2001**, *44*, 619.
- C. E. Ponnusamy, U. Fotadar, A. Spisni, D. Fiat, *Synthesis* **1986**, 48.

II. LC-MS Methods and Parameters

Equipment

LC was performed using an Agilent 1100 series HPLC equipped with an online degasser, binary pump, autosampler, heated column compartment and diode array detector. MS was performed using an Agilent XCT iontrap MSD mass spectrometer. Solvents and formic acid were acquired from Romil.

HPLC Parameters

HPLC analysis was performed by injecting 2.5 μ L of library solution onto a Waters Symmetry C18 2.1 x 150mm column and elution with a gradient of acetonitrile (0.1% formic acid) and water (0.1% formic acid) at a flow rate of 0.3mL/min.

Gradient Elution

Time (mins)	Acetonitrile (0.1% HCOOH)	Water (0.1% HCOOH)
0	5	95
30	95	5
45	95	5
50	5	95
55	5	95

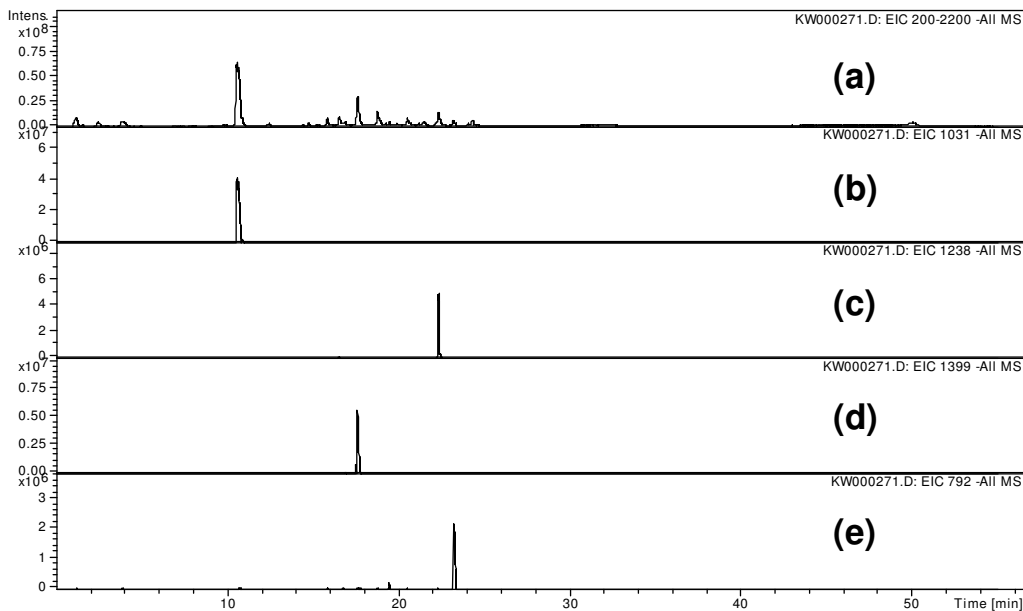
MS Parameters

Mass spectra (negative ion mode) were acquired in ultra scan mode using a drying temperature of 350°C, a nebuliser pressure of 55.00psi, drying gas flow of 12L/min, capillary voltage 4000V and an ICC target of 200,000 ions. We tuned for a target mass of 1000.

III. Base Peak and Extracted Ion Chromatograms for DCLs

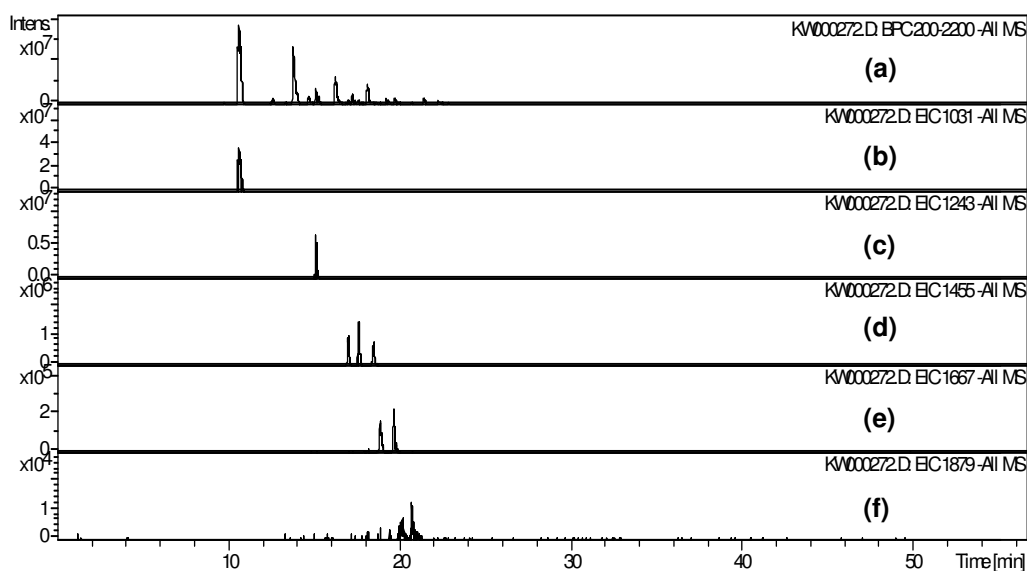
Masses were correlated to UV peaks using extracted ion chromatograms (EIC). Compounds were often detected as singly and doubly charged species. As a rule of thumb, at lower molecular weights, singly charged species dominate and at higher molecular weights doubly charged species dominate. Masses are often detected as sodium adducts.

III-1. Library of **5** and **6** (see Fig. 1 of main text)



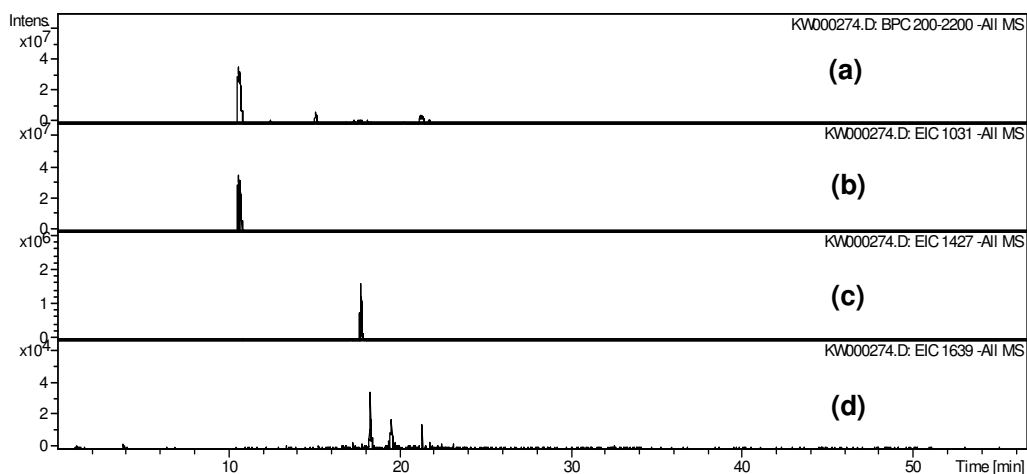
- (a) Base peak chromatogram
(b) EIC for mass = 1031 – corresponds to $(\mathbf{5})_2^{1-}$
(c) EIC for mass = 1238 – corresponds to $(\mathbf{5})_2(\mathbf{6})^{2-} + \text{Na}^+$
(d) EIC for mass = 1399 – corresponds to $(\mathbf{5})_2(\mathbf{6})_2^{1-}$
(e) EIC for mass = 792 – corresponds to $(\mathbf{5})_2(\mathbf{6})_3^{2-}$

III-2. Library of 5 and 7 (see Fig. 2 of main text)



- (a) Base peak chromatogram
- (b) EIC for mass = 1031 – corresponds to $(\mathbf{5})_2^{1-}$
- (c) EIC for mass = 1243 – corresponds to $(\mathbf{5})_2(\mathbf{7})^{1-}$
- (d) EIC for mass = 1455 – corresponds to $(\mathbf{5})_2(\mathbf{7})_2^{1-}$
- (e) EIC for mass = 1667 – corresponds to $(\mathbf{5})_2(\mathbf{7})_3^{1-}$
- (f) EIC for mass = 1879 – corresponds to $(\mathbf{5})_2(\mathbf{7})_4^{1-}$

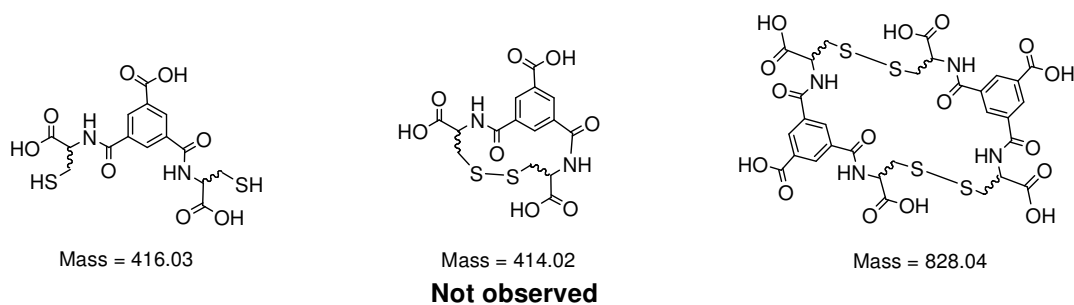
III-3. Library of 5, 6 and 7



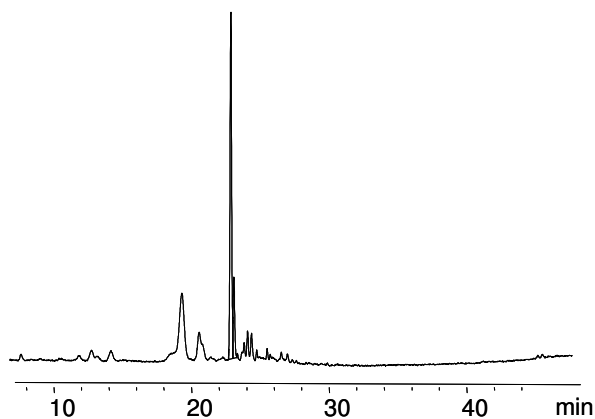
- (a) Base peak chromatogram
- (b) EIC for mass = 1031 – corresponds to $(\mathbf{5})_2^{1-}$
- (c) EIC for mass = 1427 – corresponds to $(\mathbf{5})_2(\mathbf{6})(\mathbf{7})^{1-}$
- (d) EIC for mass = 1639 – corresponds to $(\mathbf{5})_2(\mathbf{6})(\mathbf{7})_2^{1-}$

IV. Intramolecular disulfide bond experiment (see footnote 12)

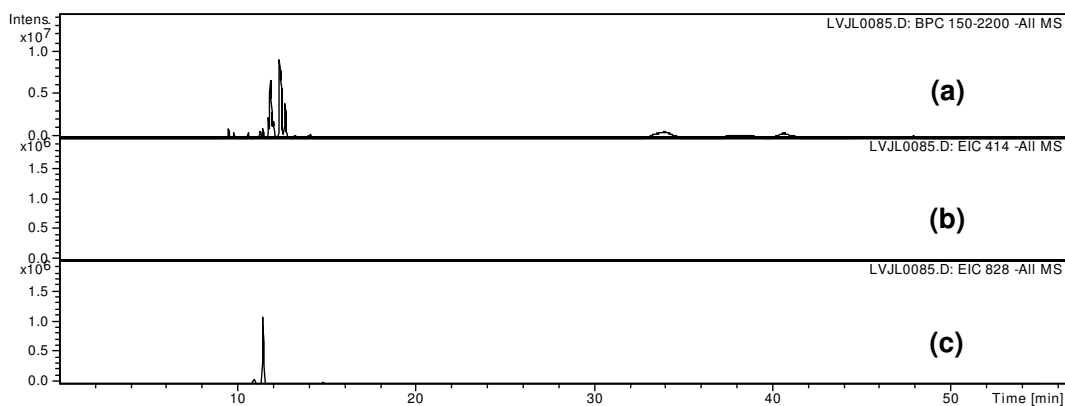
Using racemised building block containing only two cysteine units



IV-1 HPLC chromatogram



IV-2 Mass spectrometry data



- (a) Base peak chromatogram
- (b) EIC for mass = 414 – corresponds to cyclised monomer
- (c) EIC for mass = 828 – corresponds to cyclised dimer

V Additional ^1H NMR data suggesting cage structure of $(5)_2$ (see footnote 12)

500 MHz ^1H NMR of **5 (top) and $(5)_2$ (bottom) in d_7 -DMF at 300 K**

